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Note

Apolipoprotein B mRNA Editing in the Liver and Small Intestine of Rats Fed on Beet Fiber, Soy Protein, and Fish Oil

Reiko MIYAKAWA, Kei SONOYAMA,† and Takanori KASAI

Laboratory of Food Biochemistry, Department of Bioscience and Chemistry, Faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

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Apolipoprotein B mRNA editing was investigated in the liver and small intestine of rats fed on beet fiber, soy protein, or fish oil as plasma cholesterol-reducing agents. The diets had no influence on the editing in both the liver and intestine, despite their cholesterol-lowering action. The results suggest that apo B mRNA editing is not involved in the cholesterol-lowering effect of these diets.

Key words: apo B mRNA editing; plasma cholesterol; liver; intestines; rat

Apolipoprotein B (apo B) is synthesized in both the liver and intestines of mammals and serves an essential role in the assembly and secretion of triglyceride-rich lipoproteins.[1] ApoB circulates in the plasma in two molecular forms which have been referred to on a centile scale as apo B-100 and apo B-48.[2] Human apo B-100 is predominantly synthesized by hepatocytes and integrated into VLDL and LDL as their constitutional apolipoprotein. Human apo B-48, an amino terminal half-part of apo B-100, is synthesized by intestinal epithelial cells and secreted into the lymph as a component of chylomicrons.[2] Both peptides are the translational products of a single apo B gene encoding apo B mRNA that undergoes post-transcriptional conversion of cytosine at nucleotide position 6666 to uracil, thereby replacing the glutamine codon (CAA) with an inframe stop codon (UAA). This process is referred to as apo B mRNA editing.[3,4] Consequently, the translation of this apo B mRNA produces the shorter apo B-48. LDL containing apo B-100, which can be recognized by the LDL receptor, undergoes the receptor-mediated uptake at a relatively slow clearance rate (2–3 d). In contrast, apo B-48 cannot be recognized by the LDL receptors. Apo B-48-containing lipoproteins are cleared by cellular uptake through putative remnant receptors in the liver, resulting in a rapid clearance from the plasma (in a matter of minutes).[5] In contrast to humans, a part of apo B-100 mRNA is edited to apo B-48-type mRNA in rodent hepatocytes, and consequently both types of apo B are synthesized by the liver.[6] Therefore, an alteration in the efficiency of hepatic apo B mRNA editing can lead to changes in the plasma lipoprotein profile. In fact, Baum *et al.*[7] have demonstrated that the increase of hepatic apo B mRNA editing in rats fasted and refed on a high carbohydrate diet was reflected in the proportions of apo B-100 and apo B-48 in the plasma. Interaction between the LDL receptor and apo B-100 plays an important role in regulating the plasma cholesterol level in mammals. In this respect, it is likely that alteration of hepatic apo B mRNA editing and subsequent changes in the plasma lipid profile are involved in regulating the plasma cholesterol level. To date, dietary factors have been reported to regulate the plasma cholesterol level. However, it has remained unknown whether apo B mRNA editing was involved in this regulation. In the present study, we investigate the effect of diets containing either beet fiber, soy protein or fish oil on apo B mRNA editing in the rat liver and small intestine. It is well known that these diets could lower the plasma cholesterol level.[8–10]

Male Wistar rats (Japan SLC, Hamamatsu, Japan), which were 5 wk old at the start of the experiment, were housed in individual cages in a temperature-controlled (23±1°C) room with the dark period from 7:00 p.m. to 7:00 a.m. They were allowed to free access to water and to a purified control diet consisting of (as wt/wt) 25% casein, 65% sucrose, 5% corn oil, 4% mineral mixture and 1% vitamin mixture[11] prior to the experiment. This diet is used as a standard rat diet in our laboratory because we have found that it yields the maximal growth rate. After 3 d of consuming the diet, the rats were divided into four groups of 6 animals each, and each group was fed on either the control diet, or the same diet containing 15% beet fiber (Nippon Beet Sugar Co., Obihiro, Japan), 25% soy protein isolate (Fuji Seiyu Co., Osaka, Japan) or 5% fish oil (EPA-28, Nippon Suisan Co., Tokyo, Japan). The beet fiber, soy protein isolate or fish oil was added to the respective diet at the expense of sucrose, casein or corn oil, respectively. The animals had free to access to the diets and water for 14 d. On the last day of the experiment, they were anesthetized by an intraperitoneal injection of Nembutal (sodium pentobarbital, 50 mg/kg body wt; Abbott Laboratories, North Chicago, IL, U.S.A.). After a laparotomy, blood samples were collected from the abdominal aorta to determine the plasma lipid concentration. The liver was excised, immediately plunged into liquid nitrogen, and stored at −80°C for RNA extraction. Two 10-cm portions of the small intestine were excised, one at 10 cm distal to the ligament of Treitz as the jejunal segment, and the other just proximal to the ileocecal valve as the ileal segment, and the luminal contents were washed out with 5 ml of ice-cold saline. The intestinal

† To whom correspondence should be addressed. Fax: +81-11-706-2496; E-mail: ksnym@chem.agr.hokudai.ac.jp

mucosa was scraped off with a glass slide, snap-frozen in liquid nitrogen, and stored at −80°C for RNA extraction.

The plasma lipid concentrations were determined by commercially available kits.

Total RNA was isolated by ISOGEN (Nippon Gene, Tokyo, Japan) from the liver and intestinal mucosa as recommended by the manufacturer.

The endogenous apo B mRNA editing ratio was analyzed by RT-PCR coupled with a primer extension/dideoxynucleotide chain termination assay as previously described.[12] The signal intensity of the edited and unedited bands of apo B cDNA was determined by densitometric scanning. Each result is expressed as the mean±SE. A statistical comparison of the means was carried out by Student's *t*-test.

The initial body weight of the four groups was the same and averaged 143±2 g. Body weight gain per 14 d was also the same: 95.3±8, 96.2±4, 109±8 and 87.2±4 g for the rats fed on the control, beet fiber, soy protein and fish oil diets, respectively. The average daily food intake was 16.0±0.7, 16.3±0.6, 16.7±0.6 and 14.8±0.5 g by the rats fed on the control, beet fiber, soy protein and fish oil diets, respectively. The intake of the fish oil diet was significantly lower than that of beet fiber and soy protein diets ($p<0.01$).

Table 1 shows the plasma lipid concentrations in the rats fed on the four experimental diets. After 14 d of consumption, the plasma total cholesterol concentrations were significantly lower in the rats fed on beet fiber, soy protein, and fish oil diets than in those fed on the control diet. This difference was due mainly to the lower HDL cholesterol concentrations. The plasma triglyceride concentration was significantly lower in the rats fed on the fish oil diet than in those fed on the other diets.

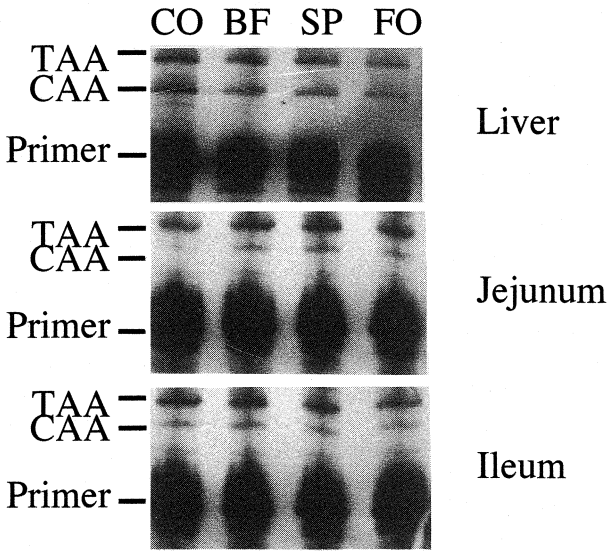

Fig. 1. Endogenous apoB mRNA Editing in the Liver and Small Intestine of Rats Fed on the Different Diets for 14 Days.

The photograph shows representative results of the primer extension assay of PCR-amplified apo B cDNA in the rat liver and small intestine. TAA and CAA represent edited and unedited apo B cDNA, respectively. CO, control; BF, beet fiber; SP, soy protein; FO, fish oil.

Table 1. Aortic Plasma Lipid Concentrations in Rats Fed on the Different Diets for 14 Days

Diet	Plasma cholesterol		Triglyceride	Phospholipid
	Total	HDL		
	(mg/dl)			
Control	96.4±3.9[a]	68.5±5.0[a]	85.8±15.0[a]	184±4[a]
Beet fiber	76.2±3.9[b]	53.0±2.7[b]	84.1±6.2[a]	161±6[b]
Soy protein	79.7±3.5[b]	52.2±1.9[b]	73.5±10.6[a]	165±6[b]
Fish oil	74.3±5.4[b]	50.3±3.5[b]	41.6±6.2[b]	137±5[c]

Values in a column not sharing a common superscript letter are significantly different ($p<0.05$). Each value is the mean±SE (n=6).

Table 2. Endogenous Apolipoprotein B mRNA Editing in the Liver and Small Intestine of Rats Fed on the Different Diets for 14 Days.

Diet	Liver	Jejunum	Ileum
		% TAA	
Control	53.0±1.53	70.7+3.75	72.4+1.45
Beet fiber	49.8±0.52	72.7+1.44	73.5±1.50
Soy protein	56.1±1.50	73.5±0.94	72.0±0.90
Fish oil	52.3±0.94	73.3±1.33	74.2±0.90
p	>0.1	>0.1	>0.1

Apo B mRNA editing is expressed as the percentage of apo B-48-type cDNA (TAA) in total apo B cDNA (TAA+CAA). Each value is the mean±SE (n=6).

Figure 1 shows the representative results of the primer-extension assay of PCR-amplified apo B cDNA in the liver and small intestine of the rats fed on the different diets for 14 d. The upper and lower bands show apo B-48-type (edited) cDNA and apo B-100-type (unedited) cDNA, respectively. The percentage of edited apo B transcripts is shown in Table 2. There was no significant difference in both hepatic and intestinal apo B mRNA editing among the groups.

In the present study, it was found that the decrease in the plasma total cholesterol concentration by the dietary beet fiber, soy protein and fish oil diets was ascribable to the decrease in the plasma HDL-cholesterol and not to the VLDL- and LDL-cholesterol concentrations. As the principle protein constituent of VLDL and LDL is apo B, it is unlikely that the plasma apo B concentrations and/or profile, *i.e.* the ratio of apo B48/apo B100, were changed by the dietary components tested in the present study. In fact, we could not observe any changes in the editing of apo B mRNA in the liver and small intestine, which should have influenced the plasma profile of apo B. Therefore, to elucidate the involvement of apo B mRNA editing in the hypocholesterolemic effect of dietary factors, further investigations will be required under the condition that the plasma VLDL- and LDL-cholesterol concentrations are lowered by the dietary factors.

The present study shows that the fish oil diet lowered the plasma triglyceride concentration, being consistent with the results in previous reports.[13–15] It has reportedly been shown that the hypotriglyceridemic effect of fish oil rich in eicosapentaenoic and docosahexaenoic acids was

due to decreased lipogenesis, increased fatty acid oxidation, and reduced VLDL secretion by the liver.[14-16] In addition, Ikeda *et al.*[17] have recently reported that dietary eicosapentaenoic and docosahexaenoic acids had different effects on the hepatic lipogenesis and fatty acid oxidation in rats. Furthermore, Brown *et al.*[16] have observed that apo B-48 synthesis was decreased in hepatocytes from fish oil-fed rats. The authors consider, however, that these decreases cannot be expected to have resulted from the decrease of apo B mRNA editing activity, since the authors did not observe any increase in the synthesis of apo B-100.[16] Thus, their suppositions could be supported by the present results showing unchanged apo B mRNA editing in the liver of rats fed on fish oil.

In conclusion, we propose that apo B mRNA editing is not involved in the cholesterol-lowering effect of beet fiber, soy protein or fish oil. Further studies will be necessary to understand the relationship between dietary circumstances and apo B mRNA editing.

Acknowledgments

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